Macrocyclic Saccharide Bundles as a New Type of Firm DNA Binders

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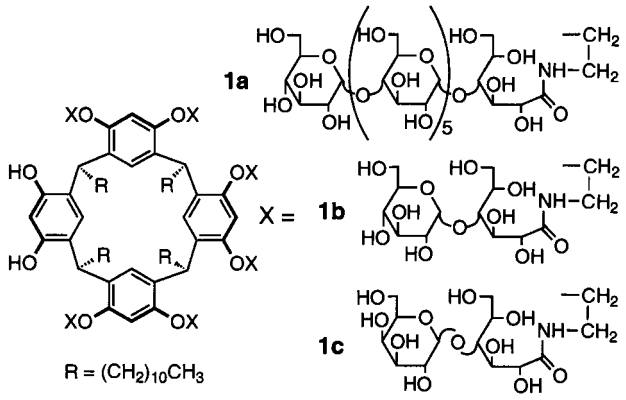
(Received December 28, 2000; CL-001172)

Double helical DNAs such as plasmid DNA pBR322 and calf thymus (CT) DNA were firmly bound to calix[4]resorcarene-based saccharide bundles, as demonstrated by the electrophoretic, circular dichroism (CD) spectroscopic, dynamic light scattering, and thermal-stability criteria. The long-chain cluster compound interacts with DNAs more effectively than a shorter one.

The characteristic structural integrity of DNAs as polyanionic and stacked heterocycles having unsaturated hydrogen-bonding sites at the periphery has led to the discovery of various DNA- and RNA-binding molecules.[1] Saccharides are a newest class of RNA-binders, although scattered information is available only for naturally-occurring particular polysaccharides.[2,3] We are interested in the bundle or cluster motif of saccharide chains as an expression of the "polymeric" nature of saccharides on the cell surfaces.[4] The present work is based on our previous finding of the remarkable saccharide-phosphate hydrogen-bonding in water, using calix[4]resorcarene-based saccharide cluster compounds.[5] We report here that double helical DNAs are firmly bound to these macrocycles, as demonstrated on all the electrophoretic, CD spectroscopic, dynamic light scattering, and thermal-stability criteria.



Octakis(maltoheptaose) bundle compound **1a**[6] was obtained from the reaction of macrocyclic octaamine with maltoheptaose lactone in a similar manner as the octakis(maltose) and -(lactose) analogues **1b** and **1c**. Electrophoresis gel shift is a convenient assay of DNA complexation. Thus, the supercoiled, closed circular, and open circular forms (forms I, II, and III, respectively) of plasmid DNA pBR322 (4361 base pairs) are rendered hardly mobile in the presence of long-chain compound **1a** at >20 μM (Figure 1, lanes 1–4). A short-chain counterpart **1b** at higher concentrations (>10 mM) is also effective in immobilization of pBR322. The effective masking of DNA charges by the uncharged saccharide clusters is remarkable.

The interactions of linear fragments of calf thymus (CT) DNA (500–2000 base pairs) can be evaluated on a number of criteria. Figure 2 shows that the CD bands of CT DNA (53 μM as base) in a typical pattern for B-type DNA duplexes[7] undergo red-shifts with concomitant reduction in the intensity of the longer-wavelength component. The intensity roughly shows a linear dependence on [**1a** or **1b**] and reaches a plateau region at [**1a** or **1b**] ≅ 30 μM, which is close to the base-pair concentra-

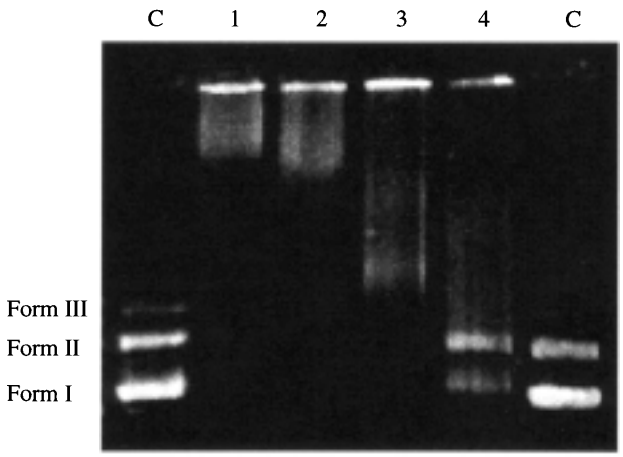


Figure 1. Gel shift assay of the interactions between pBR322 and macrocyclic saccharide clusters: Electrophoresis of pBR322 in the presence of **1a** (100, 50, 20, and 10 μM for lane 1−4, respectively); C. control. The concentration of DNA was fixed at 31 ng/μL.

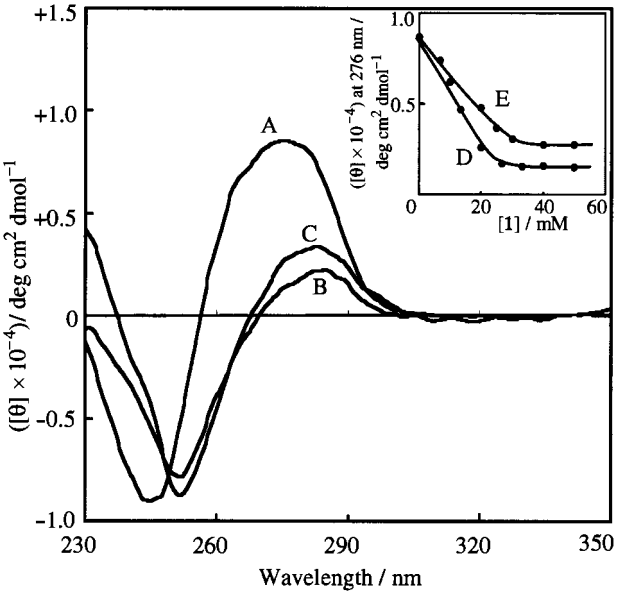


Figure 2. CD spectra of CT DNA (53 μM-base) in aqueous HEPES buffer (0.01 M, pH 7.4, μ 0.15 with NaCl) in the absence (A) and presence of macrocyclic saccharide cluster **1a** (40 μM) and **1b** (40 μM) (B and C, respectively). Inset: Correlation between CD band intensity at 276 nm and concentration of macrocyclic saccharide cluster **1a** and **1b** (D and E, respectively).

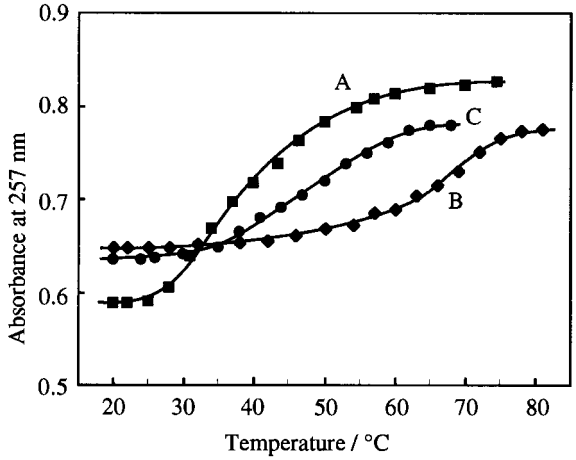


Figure 3. UV heating curves of CT DNA (45 μM-base pair) in H_2O in the absence (A) and presence of macrocyclic saccharide cluster **1a** (1.7 μM) and **1b** (1.7 μM) (B and C, respectively).

tion of the DNA (inset of Figure 2). This is a clear indication that the present saccharide-DNA interaction is fairly firm and occurs on a stoichiometric basis as regards the CD-responsible base pairs even at a micromolar concentration range. This is also confirmed from a different line of evidence. CT DNA (45 μM as base pair) "melts" i.e., undergoes a double-to-single strand transition at 38 °C. The melting curve undergoes a higher-temperature shift in the presence of host **1a** or **1b** in a concentration as low as 1.7 μM, resulting in an increase in melting point by 27 °C with **1a** or 9 °C with **1b** (Figure 3). The saccharide-promoted stabilization of the double helices vs single strands may be taken in a general context of saccharide-induced DNA aggregation. Owing to their bundle structures, the present saccharide compounds are multivalent and are potential DNA cross-linkers. Indeed, the average hydrodynamic diameter of CT DNA (95 μM as base pair) increases significantly (from 180 nm to 550 nm) in the presence of even the short-chain compound **1b** at 33 μM, as shown in the size distribution evaluated by dynamic light scattering (Figure 4).[8]

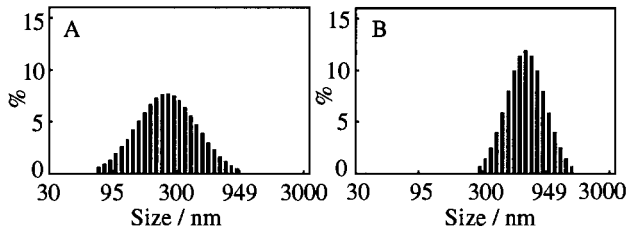


Figure 4. Evaluated size-distribution for CT DNA (95 μM-base pair) by DLS in aqueous HEPES buffer (0.01M, pH 7.4, μ 0.15 with NaCl) at 25°C: in the absence of any host (A), in the presence of **1b** (33 μM) (B).

Control experiments indicate that the lactose-derived analogue **1c** behaves similarly as the maltose counterpart **1b**, while the simple saccharides such as maltose, maltoheptaose, and dextran have no effect on the DNAs. With these in mind, the present work may be summarized that double helical DNAs are fairly strongly bound to the nonhelical saccharide bundles primarily via multivalent host-guest interactions. The long-chain cluster compound interacts with DNAs more effectively than a shorter one. Since the lactose-derived compound with terminal galactose residues is known to play a decisive role in cell targeting,[9] a promising future area is the use of this type of macrocyclic saccharide clusters as cell-specific gene carries.[10] Work is now going on along this line.

Reference and Notes

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6 Saccharide cluster **1a** was prepared by ring-opening amine-lactone reaction of calix[4]resorcarene octaamine with maltoheptaose-derived lactone in a manner similar to that applied to the synthesis of **1b**: a white solid, mp 182–186 °C (dec.), Anal. Calcd for $C_{424}H_{712}N_8O_{296}$: C, 47.78; H, 6.73; N, 1.05%. Found: C, 48.16; H, 6.73; N, 1.38%.

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